Novel Platform for Large-Scale Analysis of Brain Cell Types at Single-Cell Resolution Spanning Longitudinal Multi-Modal Measurements

Olaia F Vila[1], Nick Elder[1], Sina Farahvashi[1], Isaac Thomas[1], Josh Lamstein[1], Shreya Deshmukh[1], Teresa Ai[1], Pier Federico Gherardini[1], Gary P Schroth[1].
[1]Cellanome, Foster City, CA

INTRODUCTION

Single-cell technologies have revolutionized our understanding of cellular functions and heterogeneity. However, current high-throughput methods face limitations for the study of 1) cells inadequately identified by protein markers, 2) large or fragile cells that lose viability under harsh microfluidic conditions, and 3) cells needing disruption from their functional units for transcriptional analysis. Many brain cell types fall into one or more of these categories. To address, we developed and leveraged a high-throughput, multi-modal platform facilitating the longitudinal (days to weeks) study of individual cells, co-cultures, or neuronal networks without enzymatic perturbation. Single cells or co-cultures can be encircled within CellCage™ enclosures, called CCEs, in which cells can be cultured and imaged for days to weeks to evaluate morphological changes over time due to differentiation, maturation, or proliferation. After, they are processed within CCEs for downstream sequencing (mRNA, CRISPR guides). Notably, this enables the capture of transcriptomics linked to longitudinal data around disease-relevant cellular functions, including phagocytosis, neuron-astrocyte interactions, and calcium signaling from cells that are functionally intact at the time of processing. This technology can become a key tool for unlocking physiologically relevant insights to advance the study of neuro-immunological and neurodegenerative diseases.

A NOVEL MICRO-3D PRINTING TECHNOLOGY ENABLES HIGH-THROUGHPUT EVALUATION OF CELL PHENOTYPES AND TRANSCRIPTOMES

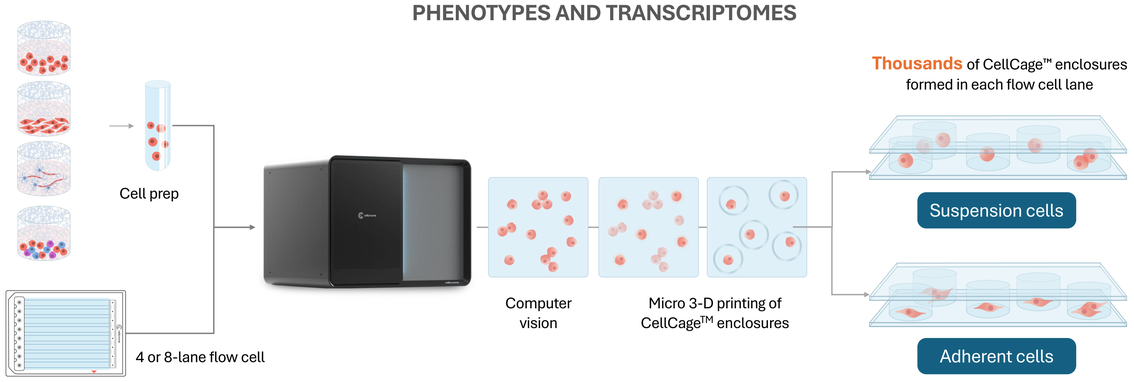

Fig 1. Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. Tens of thousands of cells in suspension are mixed with hydrogel precursor and loaded on a 4 or 8-lane flow cell. Positions of cells are identified and CellCage™ enclosures are generated around cells with light-guided polymerization in an automated fashion. CellCage™ enclosures are permeable to reagents (e.g., small molecules, antibodies) enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells. Cells can be lysed within CellCage™ enclosures to generate barcoded cDNA for downstream library prep and sequencing off the instrument.

WORKFLOWS FOR STUDYING NEURONS IN CELLCAGE™ ENCLOSURES

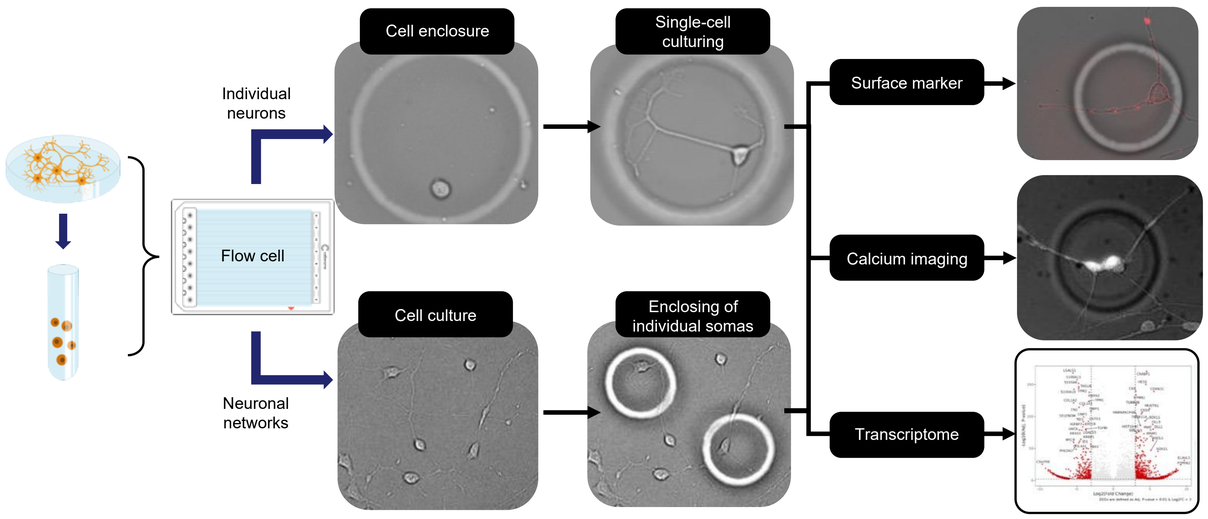

Fig 2. Generation of CellCage™ enclosures around whole neurons or somas in networks. (TOP) Suspension neurons are loaded in the instrument, where they are mixed with hydrogel precursor and loaded into a flow cell lane. Cells are automatically detected and CellCage™ enclosures are created around individual neurons. Neurons are then incubated to allow for attachment and neurite extension within the enclosure. (BOTTOM) Suspension neurons are loaded into a flow cell lane and incubated to allow for attachment and neurite extension. After days / weeks, hydrogel precursor is loaded in the lane, soma are automatically detected and CellCage™ enclosures are created around them. After they are enclosed, neurons can be imaged over time, using brightfield and fluorescent imaging. At the end of the experiment, the transcriptome of these neurons / somas can be isolated and linked to the imaging data.

INTEGRATED CALCIUM IMAGING AND TRANSCRIPTOME DATA FROM SINGLE ASTROCYTES IN CCEs

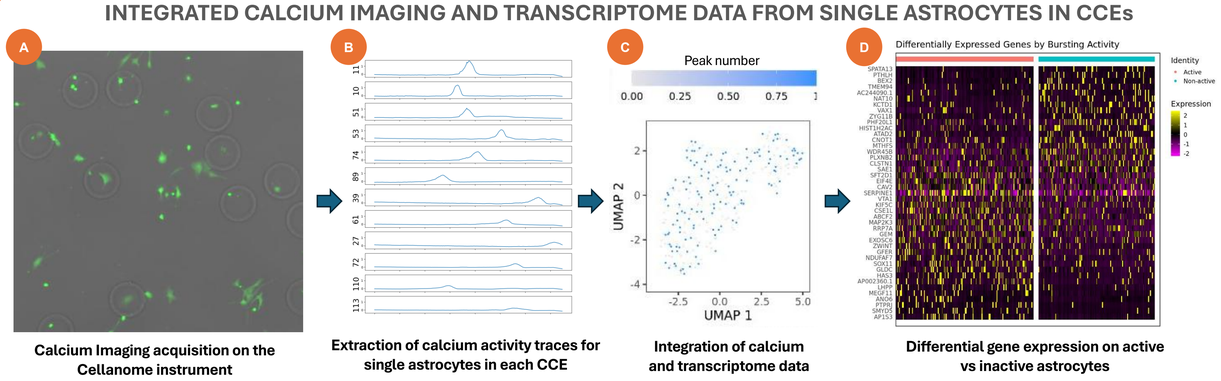

Fig 3. Acquisition of calcium imaging and transcriptome data from single astrocytes in CCEs. (A) iPSC-derived astrocytes were enclosed in CCEs and imaged using Fluo4-AM calcium dye. Each FOV was imaged at 1.3 Hz for 5 minutes. (B) Calcium imaging data was processed to extract activity traces for single astrocytes in each CCE. (C) After calcium activity was measured, scRNA-seq data was collected from the same single cells, enabling linked scRNAseq and calcium activity data to be collected from the same cell. (D) Differential gene expression analysis was performed comparing active vs inactive astrocytes.

NEURON-ASTROCYTE CO-CULTURES IN CELLCAGE ™ ENCLOSURES

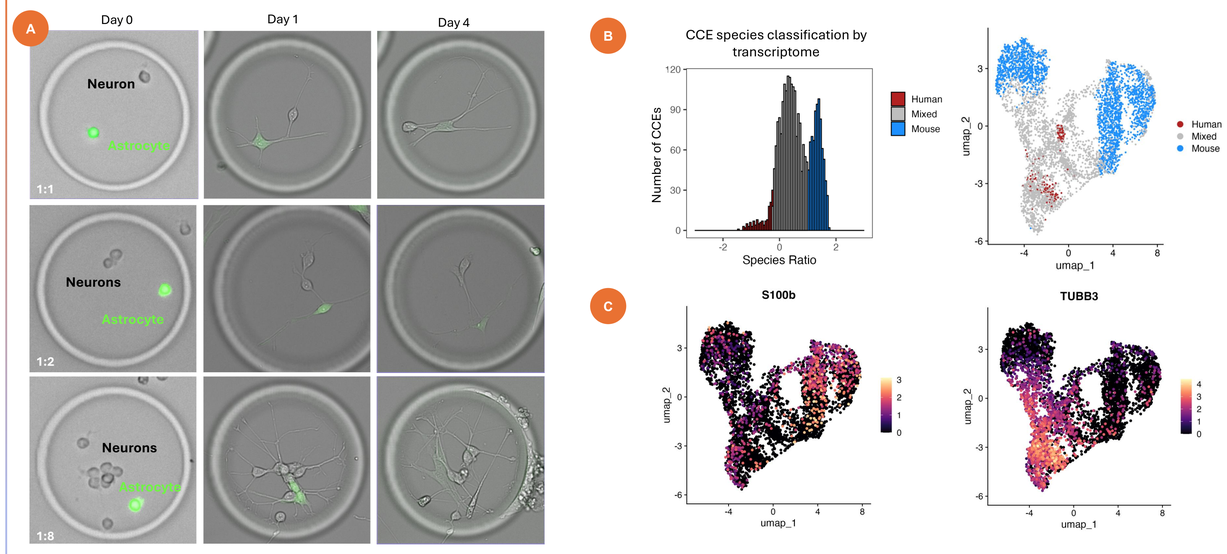

Fig 4. Linked image and transcriptome analysis astrocyte-neurons co-cultures. (A) Mouse astrocytes were labeled with CFSE dye (green) and mixed with human iPSC-derived neurons before loading them into our flow cell. CCEs were formed using fluorescent targeting of astrocytes. Single astrocytes were encircled with a Poisson distribution of neurons, as "passenger cells," enabling co-cultures to span a distribution of astrocyte:neuron ratios from 1:1 to 1:8. (B) After 4 days in culture, cells were lysed and barcoded transcriptomes were isolated from each CCE. Transcriptome data can be matched to imaging data, resulting in a linked, multi-modal dataset. Gene expression analysis enables deconvolution of astrocyte and neuron data, using mouse and human genes. (C) Mouse transcripts express astrocyte markers (S100b, left), while human transcripts include neuronal makers (TUBB, right).

PHAGOCYTOSIS ASSAY ON MICROGLIAL CELLS

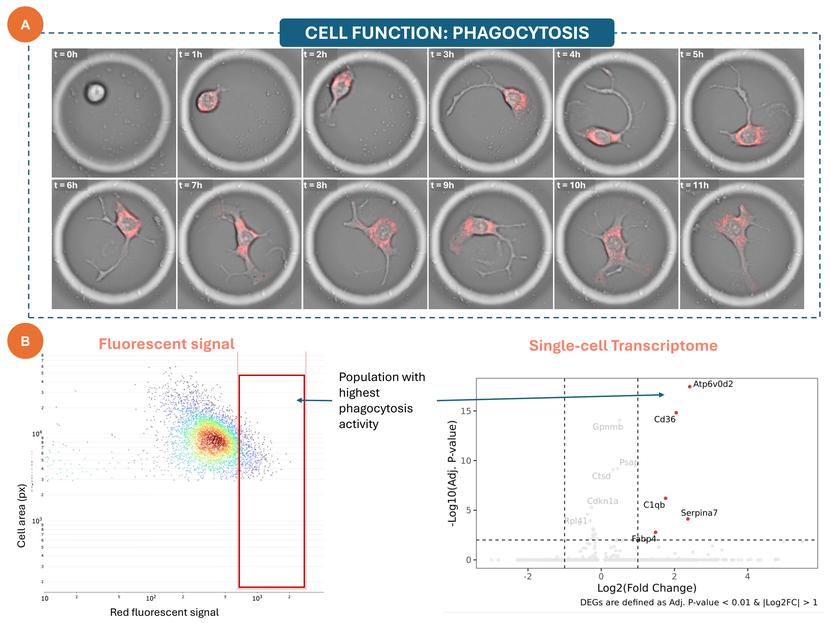

Fig 5. Correlation of phagocytosis activity and transcriptome on BV2 microglial cells. (A) Time-lapse of a single BV2 microglial cell phagocytosing pHrodo™ BioParticles™. CellCage™ enclosures were formed around single microglial cells in the presence of red E. coli pHrodo BioParticles. Images were acquired every hour over a 12h period. Increase of red signal shows phagocytosis of the particles. (B) High-throughput analysis of phagocytosis activity and single-cell transcriptome. Microglial cells with the highest phagocytosis activity show overexpression of genes related to phagocytosis and complement signaling (Cd36, C1qb), lipid transport and oxidative stress regulation (Serpina7, Fabp4) and lysosomal function (Atp6v0d2).

NEUROSPHERE CULTURE IN CUSTOM CELLCAGE™ ENCLOSURES

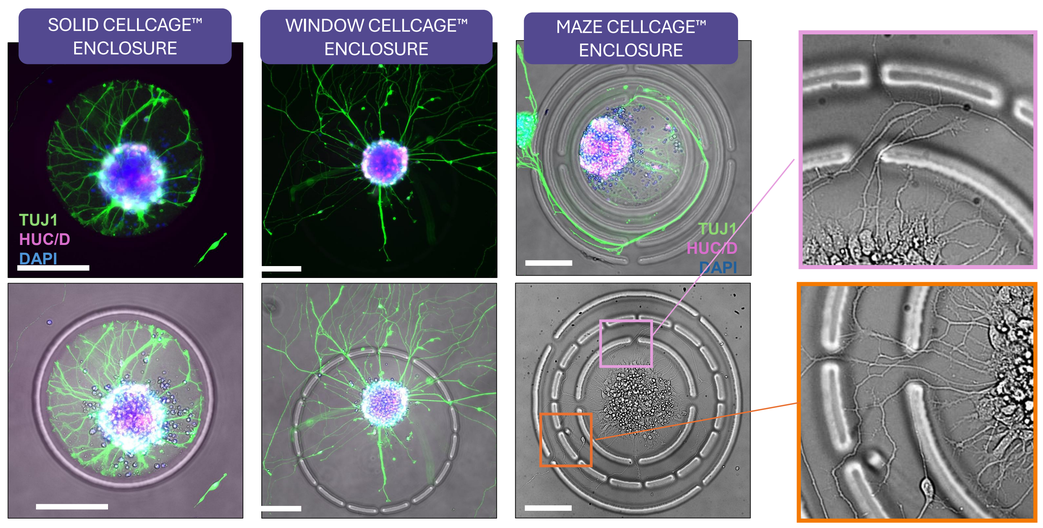

Fig 6. CellCage™ enclosures support the culture of neurospheres. V2a neurons were dissociated into single cells and seeded into AggreWells at a density of 100-200 cells per well. After 48 hours, neurons formed compact neurospheres. Neurospheres were then loaded into a flow cell lane and CellCage™ enclosures formed around individual neurospheres. CellCage™ enclosures can be modified to have openings of configurable sizes and / or layered in different patterns to restrict or permit neurite outgrowth, allowing spatially restricted neurospheres to contact one another or to test neurite pathfinding in response to external cues, such as added morphogens. Scale bar:130 µm. Close-up images showcase growth cone-led axonal growth through CellCage™ enclosure openings.